A Novel Heparinized Chitosan/PLAGA Scaffold for Bone Tissue Engineering

Tao Jiang [a], Cato T. Laurencin [a,b,c]
Department of Chemical [a], Biomedical Engineering [b] and Orthopaedic Surgery [c], University of Virginia, Charlottesville, VA 22904

Introduction: Biomaterials represent a major component in many tissue engineering approaches. In recent years, with further understanding of cell-biomaterial interaction, biomaterial science has advanced to design substrates with elegantly engineered surface and bulk material chemistry, tailored surface topography and bulk architecture to finely direct cell and tissue growth. We have previously reported novel three-dimensional (3-D) chitosan/poly(lactide-co-glycolide) (PLAGA) matrices with excellent mechanical properties suitable as scaffolds for bone tissue engineering applications [1]. In addition, heparin, a linear highly sulfated glycosaminoglycan consisting of sulfated repeating disaccharide unit, has long been used in the clinic as an anticoagulant and is also widely recognized to be an important bioactive molecule capable of interacting with heparin-binding growth factors [2]. The aim of the present study was to develop and evaluate novel heparinized chitosan/PLAGA scaffolds for bone tissue engineering.

Materials and Methods: 3-D composite chitosan /PLAGA sintered microsphere scaffolds were fabricated as previously described [1]. Scaffolds were then surface protonated by treating with 0.5% acetic acid, and subsequently immersing in 1ml of 4μg/ml or 40μg/ml heparin solutions for 2 hours. The amount of immobilized heparin was estimated using a Chromogenix Coatest® heparin kit (DiaPharma, West Chester, OH). MC3T3-E1 osteoblast-like cells were seeded on pure PLAGA scaffolds (PLAGA), non-heparinized chitosan/PLAGA scaffolds (chitosan/PLAGA), and heparinized chitosan /PLAGA scaffolds (chitosan/PLAGA 4ug heparin/40ug heparin) at a density of 5×10^4 cells/scaffold. Cells were cultured in α-minimum essential medium supplemented with 10% FBS, 1% antibiotics, 3mM β-glycerophosphate and 10μg/ml ascorbic acid. At day 14, cell-scaffold constructs were stained with DAPI for cell nuclei and TRITC-conjugated phalloidin for cytoskeletal actin. Alkaline phosphatase (ALP) activity of cells was measured with an ALP substrate kit (Bio-Rad, Hercules, CA). Osteocalcin expression in cell culture media was evaluated using a mouse osteocalcin ELISA kit (Biomedical Technologies Inc., Stoughton, MA). Statistical analysis was performed using a one way analysis of variance (ANOVA) with Tukey test.

Results and Discussion: It has been found that approximately 1.68 ± 0.05μg or 14.07 ± 4.80μg heparin was immobilized onto chitosan/PLAGA scaffolds after treating with 4μg/ml or 40μg/ml heparin, respectively. At day 14, cells completely covered the surface and pores of PLAGA scaffold (Fig. 1a). Heparinized chitosan/PLAGA scaffolds also supported cell proliferation, and as indicated in Fig. 1b cells migrated toward the gaps between microspheres. The actin cytoskeleton was well organized and distributed within the 3-D structures of scaffolds (Fig. 1a and 1b). However, compared to chitosan/PLAGA scaffold, heparin modification tended to increase the alkaline phosphatase activity of cells after 21 days of culture (Fig. 2) indicating enhanced cell differentiation. This was further corroborated by osteocalcin (OCN) expression of cells. At day 7, it was found that the OCN expression of cells on chitosan/PLAGA 4μg heparin scaffold was significantly higher than chitosan/PLAGA, chitosan/PLAGA 40μg heparin and PLAGA scaffolds. At day 14, cells on chitosan/PLAGA 4μg heparin scaffold showed significantly higher OCN expression level than chitosan/PLAGA 40μg heparin and PLAGA scaffolds. At day 21, cells on chitosan/PLAGA 4μg heparin scaffold continued to show significantly higher OCN expression level than chitosan/PLAGA scaffold. The mechanism underlying this response of MC3T3-E1 cell differentiation to heparin is yet unclear and may be due to specific cell-matrix interactions.

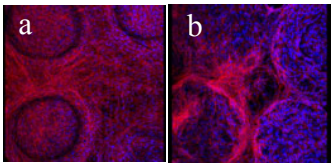

Fig.1. Immunofluorescent staining for cell nuclei (blue) and actin cytoskeleton (red) on (a) PLAGA scaffold; (b) chitosan/PLAGA 4μg heparin scaffold.

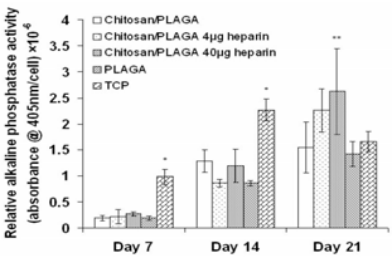

Fig. 2. ALP activity of MC3T3-E1 cells. (*): $p < 0.05$ as compared to other groups; (**): $p < 0.1$ as compared to PLAGA scaffold.

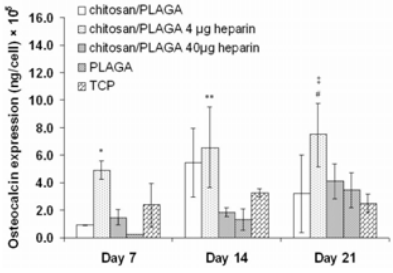

Fig. 3. OCN expression levels in media. (*): $p < 0.05$ as compared to other groups; (**): $p < 0.05$ as compared to chitosan/PLAGA 40 μg heparin and PLAGA scaffolds; (#): $p < 0.05$ as compared to TCP; (‡): $p < 0.1$ as compared to chitosan /PLAGA scaffold.

Conclusion: Chitosan/PLAGA scaffolds showed the capability to be functionalized with heparin. Immobilized heparin enhanced osteoblastic cell differentiation in a dose-dependent manner. Future research will focus on complexing heparin-binding growth factors onto the bioactive heparinized chitosan/PLAGA scaffolds.

References

1. Jiang T., et al. Biomaterials. 27: 4894 (2006)
2. Capila I., et al. Angew. Chem. Int. Ed. 41: 390 (2002)